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Hadsbjerg, Johanne; Fahnøe, Ulrik; Belsham, Graham; Rasmussen, Thomas Bruun

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Antivirals, vaccines and
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Sequence adaptation during growth of modified classical swine fever viruses in cell culture

Johanne Hadsbjerg, Ulrik Fahnøe, Graham J. Belsham, Thomas Bruun Rasmussen

¹*National Veterinary Institute, Technical University of Denmark, Lindholm, Kalvehave 4771, Denmark*

Classical swine fever (CSF) is an economically important, highly contagious porcine disease caused by classical swine fever virus (CSFV). The 5'-untranslated region (5'UTR) of CSFV contains an internal ribosomal entry site (IRES) directing the cap-independent initiation of protein synthesis. However, rather little is known about the effects of mutations within the 5'UTR on virus viability and growth. IRES mutants containing modified stem2 structures within the pseudoknot (domain III_f) have been described previously (Friis et al., 2012). Viruses containing these mutations were examined for sequence adaptations during growth within infected cells. The mutant viruses were serially passaged in porcine PK15 cells and the 5'UTR of the CSFV RNA was amplified by RT-PCR from selected passages (P-5, P-10, P-20) and sequenced. The 5'UTR sequence of the rescued parental virus (vPader10) remained stable but some adaptations occurred within the pseudoknot region of the mutant viruses, which appeared to stabilise the mutant IRES structure. The identified adaptations within the IRES region were confirmed by next generation sequencing (NGS) analysis of the complete viral genomes. In addition to the stabilised IRES structures, a single nt sequence change (G to A at nt 11,864) within the coding region for the NS5B protein (the RNA polymerase) was highly represented after 5 passages (>90%) in the virus population and by P-10 was present in essentially 100% of the viral RNA molecules. This demonstrates a strong selection pressure for this change which returns the cDNA clone derived vPader10 sequence to the consensus sequence of the Paderborn virus (#AY072924).

Friis MB, Rasmussen TB & Belsham GJ. (2012). Modulation of translation initiation efficiency in classical swine fever virus. *J. Virol* 86, 8681-8692